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Electrochemical structure of the crowded cytoplasm

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The current view of the cytoplasm as a 'bustling and well-organized metropolitan city' raises the issue of how physicochemical forces control the macromolecular interactions and transport of metabolites and energy in the cell. Motivated by studies on bacterial osmosensors, we argue that charged cytoplasmic macromolecules are stabilized electrostatically by their ionic atmospheres. The high cytoplasmic crowding (25–50% of cell volume) shapes the remaining cell volume (50–75%) into transient networks of electrolyte pathways and pools. The predicted 'semiconductivity' of the electrolyte pathways guides the flow of biochemical ions throughout the cytoplasm. This metabolic and signaling current is powered by variable electrochemical gradients between the pools. The electrochemical gradients are brought about by cellular biochemical reactions and by extracellular stimuli. The cellular metabolism is thus vectorial not only across the membrane but also throughout the cytoplasm.

Electrolyte pathways and cytoplasmic ionic strength

In the past decade, the traditional view of the cytoplasm as a bag of randomly diffusing enzymes has been replaced by a model in which a 'collection of molecular machines' [1] work together as a 'bustling metropolitan city' [2]. The molecular machines are supramacromolecular complexes of different proteins, proteins and DNA, and proteins and RNA, which emerge and disappear in the cytoplasm in a well-orchestrated and predictable manner during cell growth. Although microbiology textbooks generally describe the prokaryotic cytoplasm as an unstructured medium comprising macromolecules and low molecular weight metabolites including simple ions, increasing evidence suggests that this view is incorrect. Instead, the cytoplasm is a highly anisotropic and structured environment, in which many proteins carry out their functions as multimeric complexes at specific subcellular locations and at specific times during cell growth [3–5].

In vivo mobility measurements show that large multimeric protein complexes are unable to diffuse through the cytoplasm [6]. The translational motion of such large complexes as, for example, pyruvate dehydrogenase, and others, such as DNA–protein complexes and ribosomes, might be very slow and governed by mechanisms other than random thermal diffusion. These emerging views of the spatial and temporal order in the bacterial cell raise new questions. Which physicochemical forces maintain the stability of such time-evolving structures? What are the physicochemical mechanisms that control the flow of materials, energy and information?

Current proposals that deal with these questions draw on concepts from physical chemistry, cell biology and network engineering. These proposals include the effect of crowding on interactions of macromolecules in the cytoplasm [7–11], possible cytoplasmic 'phase separations' [12], the entropically driven order arising from high concentrations of particles [13,14], the concept of the 'metabolon' and of 'metabolite channeling' [15–18], the concept of 'hyperstructures' [19] and the current advent of modular and network cell biology [20–22]. Another view on the origin of cytoplasmic order stems from the 'dissipative structures' of Prigogine's order out of chaos theory [23]. In spite of these conceptual advances, the physicochemical mechanisms that organize the cytoplasmic macromolecules for their biological tasks remain to be uncovered.

We think that research into the responses of cells to hyperosmotic conditions can lead to insights into the macromolecular structure of the cytoplasm. Extrapolating from our work on bacterial osmosensors [24,25], we propose that the following phenomena configure the cytoplasmic macromolecules to perform their biochemical and physiological functions. (i) Negatively charged macromolecules and their complexes mutually repel through the 'screened electrostatic forces' of classical physical chemistry to retain their individuality [26–30]. (ii) The high crowding of cytoplasmic macromolecules shapes the remaining cytosol into a system of electrolyte pools and pathways. (iii) The charges on the surfaces of these pathways act as switches [28], which control the cytoplasmic transport of ions. (iv) The pools have unequal 'bulk' concentrations of ionic metabolites, the gradient of which drives their electrochemical transport through the pathways. This model could also apply to the interiors of some eukaryotic cells [1,2,31,32] and their organelles, but our focus here is on the crowded cytoplasm of prokaryotes [3–6].

Clues from bacterial osmosensors

Recently, there has been significant progress in our understanding of how osmoregulatory transporters

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become activated in bacterial membranes [24,25,33–37]. Osmoregulatory transporters import osmoprotectants into the cell under hyperosmotic stress and thereby assist in the survival of bacteria. The transporters OpuA, ProP and BetP from *Lactococcus lactis*, *Escherichia coli* and *Corynebacterium glutamicum*, respectively, have been found to be non-homologous proteins that differ in their use of energy for osmoprotectant transport (ATP hydrolysis for OpuA, H^+ and Na^+ electrochemical gradients for ProP and BetP). Notably, all three transporters are activated by an increase in the concentration of lumenal ions when the systems are reconstituted and energized in proteoliposomes [24]. For BetP and OpuA, the activation is a strong function of the anionic lipid content (charge density) of the membrane.

These observations suggest that there is a common physicochemical mechanism of osmosensing, which for OpuA has been modeled as an on–off electrostatic switch that assumes electrostatically locked or thermally relaxed conformations. This on–off activation has been found to correlate with the theoretically predicted transitions in ionic clouds around charged surfaces [24]. Thus, both experiment and theory point towards the notion that electrostatic forces play a principal role in the conformational states of osmosensing membrane proteins. Below, we generalize this conclusion to the interactions of macromolecules both within the cytoplasm and with the membrane and suggest an electrochemical model of cell structure.

Electrostatic stabilization of the cytoplasm

How are the cytoplasmic macromolecular surfaces stabilized against haphazard aggregation? How does order emerge from the diffusional chaos [38]? We assume that positive macromolecular charges (and small cations) neutralize lipid and macromolecular negative charges only partially. This partial neutralization yields an overall negative stabilization of the cytoplasmic macromolecules. *In silico* analysis of prokaryotic genomes shows that several proteins have an isoelectric point (p*I*) of <7, which strongly suggests that their surfaces are anionic at the ambient pH in the cell. Examples include $>70\%$ of all proteins of the well-studied model organisms *E. coli* and *Bacillus subtilis*, and $>90\%$ of the most abundant proteins of these two organisms [39,40]. Among the most abundant proteins are the enzymes of the glycolytic pathway, the citric acid cycle, the aminoacyl-tRNA synthetases and the translation elongation factors of the translational apparatus [41]. In general, these proteins are highly conserved proteins that have anionic surfaces in many prokaryotic species.

The negative charges arise chiefly from glutamates and aspartates in proteins and the phosphates of DNA and RNA. The surface positive charges arise mainly from lysine, arginine and histidine. The macromolecules associate to form supramacromolecular complexes by electrostatic attractions – for example, positively charged protein residues become neutralized by associating with membrane lipids, DNA or highly anionic proteins – which often act in concert with hydrophobic interactions [42,43]. Additional stabilization comes from non-charged amino acids, the hydroxyl and amide groups of which remain hydrogen-bonded to water molecules after the proteins have folded, associated with the membrane or become a subunit in the supramacromolecular complexes.

The cytoplasmic surfaces thus remain negatively charged and 'watery' [44]. They are bathed in concentrated electrolyte solutions containing many small metabolites that are usually ionic such as phosphorylated sugar derivatives of metabolic and signaling pathways, Krebs cycle intermediates, ATP, ADP and c-AMP, as well as simple ions including Cl^-, $H_2PO_4^-$ and HPO_4^{2-} and K^+. The main anion that is measured in *E. coli* is glutamate, whereas glutamate, phosphate and sugar-phosphates dominate in *L. lactis*; generally, K^+ is the dominant cation in bacteria [35,45]. The divalent cations Mg^{2+} and Ca^{2+}, or larger polyvalent organic polyamine cations, are usually sequestered as complex ions with highly negative phosphate functionalities.

The above description of the overwhelmingly coulombic character of the cytoplasmic milieu invokes the role of electrostatic interactions. According to classical physical chemistry, negatively charged surfaces induce the formation of ionic atmospheres in the surrounding solution by attracting cations and repelling anions [26,27]. The forces that arise from the overlapping of the ionic atmospheres of two or more charged surfaces are usually referred to as 'screened electrostatic forces', because the ionic atmospheres screen (i.e. reduce) the range of classical coulombic forces. Our focus on screened electrostatic forces does not neglect hydration effects, which are taken into account by the dielectric constant of water [46,47]. In Box 1, we define and discuss further these screened electrostatic forces.

We must briefly consider the scales of time and size in relation to the cytoplasmic structure. The macromolecules and their complexes diffuse on an approximate timescale of milliseconds (and longer), whereas water, ions and low molecular weight metabolites diffuse on a timescale of microseconds [3,4,6,48]. A hypothetical millisecond snapshot reveals only the crowded surfaces [5], although the low molecular ions and metabolites are in quasi-equilibrium with the charged surfaces (i.e. thermally smoothed-out ionic clouds for ions and the dielectric constant for water molecules). As an approximation, we divide the physicochemical cytoplasmic phenomena into only two spatial and temporal hierarchies: the bigger (3–30 nm) and slower diffusing (millisecond-and-longer) macromolecular complexes, which are pseudo-equilibrated with the smaller (0.1–3 nm) and faster diffusing (microseconds-to-milliseconds) aqueous ionic metabolites. Our 'structure' of the anionically stabilized cytoplasm is then transient and is unlikely to persist throughout the volume of the whole cell. Different parts of the cytoplasm might have different short-lived structures (such as modules [20] or hyperstructures [19]) that perform different physiological functions. It remains unclear how to define cytoplasmic macromolecular hierachies that are essential for cell growth and replication (Box 2).

Are screened electrostatic forces relevant?

The screened electrostatic forces are sometimes considered to be too short-range owing to the high ionic

Box 1. Electrostatic stabilization of charged surfaces

The electrostatic stabilization of charged surfaces is described by an 'ionic cloud', a concept that is applicable to both electrolyte solutions [27] and charged colloids [26]. For low potentials, the unequal distribution of anions and cations is described by the linear Poisson–Boltzmann equation, which defines the Debye's length, $1/\kappa$, as follows:

$$1/\kappa = \left[\frac{\varepsilon_0 \varepsilon kT}{(n_{0+}z_+^2 + n_{0-}z_-^2)e^2}\right]^{1/2}$$

The denominator expresses ionic strength, where e is the electronic charge, z are ionic valencies, and n_0 is the bulk ionic concentration. The nominator contains the thermal energy, kT, multiplied by the vacuum and solvent permittivities, $\varepsilon_0\varepsilon$. The Debye's length locates the maximum excess charge density of the ionic cloud, which gets closer to the surface with the square root of increasing ionic concentration.

At higher electrostatic potentials, the nonlinear Poisson–Boltzmann equation becomes theoretically inconsistent [26–30], because the potential and charge distributions become non-Maxwellian. The theory of Maxwellian switches [28] preserves the electrostatic consistency by defining co-ion exclusion boundaries that exclude co-ions from spaces where their repulsive energy would be higher than their thermal energy. The simplest switching prediction (the appearance–disappearance of the co-ion exclusion boundary) inversely relates the charge density, σ_0, and the Debye's length, $1/\kappa$, as follows:

$$\sigma_0\left(\frac{1}{\kappa}\right)\frac{1}{\varepsilon_0\varepsilon} = \frac{kT}{z_-e} = \psi_0$$

This relationship applies to a single negatively charged surface at a surface potential of ψ_0; modified switching conditions apply to two interacting surfaces and to specific interactions of cations. Importantly, the theoretically computed co-ion exclusion distance must be added to the Debye's length to estimate correctly the increased range of screened electrostatic forces [28–30] when the ionic cloud 'switches' to higher potential distributions.

Box 2. Outstanding questions

Can specific (macro)molecular interactions be neglected?
The crowding interactions of macromolecules have been considered most often as very large deviations from the ideality of dilute solutions arising from the large size of the macromolecules [9,10] or simply as non-interacting 'hard' particles [13,14]. In such models, the only interactions are non-specific infinite repulsions on thermal collisions (hard non-bonding electron repulsions). We do not think that such a model can explain the order and structure of the cytoplasm, although the crowding and the van der Waals shape of the charged macromolecules define the complementary topology of the electrolyte pathways and pools.

What is the role of attractive physicochemical forces?
Our model is based on charged particles of arbitrary shape in electrolyte solution. We have not explicitly considered attractive interactions (e.g. electrostatic, hydrophobic, hydrogen bonding and van der Waals) that could 'close' the pathways, for example, by causing the pathways to become narrower and perhaps to collapse under hyperosmotic stress. The attractive physicochemical forces are undoubtedly involved in the formations and reconfigurations of pathways in the putative hyperstructures or modules (25–100 nm), as well as in the assembly of smaller molecular machines.

Is cytoplasmic structure maintained by large rates of energy consumption?
In principle, we can calculate the repulsions between the surfaces of a pathway that are equilibrated with two pools at the same electrochemical potential (i.e. that have no difference in concentrations of charged species of the same kind). If the attractive forces (see above) are strong enough, then the pathway will collapse. When metabolic reactions change the concentration of ionic species in one of the pools and an electrochemical gradient develops along the pathway, will the pathway be forced open? If yes, then the 'dynamic structures' of Prigogine's order out of chaos theory, maintained by the electrochemical energy dissipation, might be applicable. These questions will be best answered by experiment, because the short-range attractive forces (e.g. the surface crystallization and dehydration of cations between two negatively charged surfaces, hydrophobic interactions and the dynamics of hydrogen bonding) are not well understood theoretically.

How do we define spatial and temporal hierarchies?
We have rather roughly divided the cytoplasmic phenomena into two spatial and temporal hierarchies: the large macromolecules moving slowly and the pseudo-equilibrated low molecular metabolites. We realize that the extremely wide molecular weight distributions of the cytoplasmic macromolecular complexes might require a model with a finer division of the cytoplasmic hierachies. First, however, it will be necessary to equate cytoplasmic and membrane macromolecular complexes with known biochemical and physiological functions that are essential for maintaining life. Their spatial and temporal interactions will then enable us to construct an electrochemical wiring diagram of the cell. Such a diagram will depend on the chemical composition of the extracellular environment and on other physicochemical variables that are external to the cell.

strength of the cytoplasm and hence of little significance. We think that this view is mistaken if the severe crowding conditions in the cytoplasm are considered. An order-of-magnitude estimate of the relevance of the electrostatic forces can be obtained by comparing the surface-to-surface distance of cytoplasmic macromolecules with the Debye's length (Box 1). For example, in a prokaryotic cell of 1000 nm in diameter that is filled with ~2 million charged 'spherical particles' of 5 nm in diameter (representing a volume fraction of 25%), the shortest surface-to-surface distance is ~1.4 nm (assuming a simple cubic lattice). The Debye's lengths of approximately 0.7 nm at an ionic strength of 0.20 M extend from the surface of each particle; hence, they overlap for two particles at approximately the midway of the shortest surface-to-surface distance of 1.4 nm. Clearly, in this case the screened electrostatic forces must be operational.

In osmotically stressed *E. coli* cells (1.0 Osm of medium osmolality), the cytoplasmic concentration of potassium glutamate, the most abundant osmolyte, is increased to >0.6 molal and the concentration of macromolecules is almost doubled owing to a decrease in cytoplasmic volume [45]. Under these conditions, the Debye's length is ~0.35 nm. Using the same crowding calculation as above, at a 50% volume fraction of 5-nm spheres, the surface-to-surface distance becomes 0.08 nm (i.e. the spheres are nearly touching!). For 20-nm particles (similar to ribosomes), the surface-to-surface distance becomes 0.31 nm, which is comparable to a single Debye's length of 0.35 nm. In addition, at higher surface potentials the predicted co-ion exclusion distances must be added to the Debye's length (Box 1). These 'back-of-the-envelope' estimates are admittedly oversimplified, because there is an ever-changing distribution of sizes and charge densities of the macromolecular complexes. Nevertheless, they show that screened electrostatic forces undoubtedly

stabilize the cytoplasm against random collapse, particularly under hyperosmotic conditions.

Electrolyte pathways 'wire' the cytoplasm

The cytoplasmic macromolecules account for a ~25–30% of the volume of a normal prokaryotic cell; in an osmotically stressed cell, they can occupy a volume fraction of up to 50% [45]. Although the chemical structures, the associations, and the binding and catalytic properties of these macromolecules have been well studied over the past 50 years, the contents of the remaining cytosolic volume of 50–75% have received far less attention. This volume seems to be regarded as some undefined space through which metabolites and macromolecules diffuse to find, by trial and error, where their action might be required. The crowding of the macromolecules suggests that this volume should be considered as a complementary distribution of pools filled with water, ions and low molecular weight metabolites that are interconnected by a system of electrolyte pathways. The boundaries of these pools and pathways are formed by the charged surfaces of the cytoplasmic macromolecules enclosed by the charged membrane. Such a model has two consequences.

First, there is no cytoplasmic 'bulk' concentration of ions and metabolites (as is often assumed in biophysical models and in the design and interpretation of *in vitro* experiments); rather, each pool has its own bulk concentration of ions and metabolites. Therefore, the electrochemical gradients between the pools provide the force to transport ionic metabolites throughout the cytoplasm and through the membrane. The distribution of the electrolyte pools with their varying ionic content defines a time-dependent average that represents the cytoplasmic ionic strength. Such an average has no physical meaning *in vivo*, although it is a valid quantity *in vitro*, where macromolecules are not crowded and the ionic content is independent of time.

Second, the electrochemical pools are interconnected by electrolyte pathways (or gaps) through which ions and metabolites are either transported or prevented from being transported (see 'Maxwellian switches' below). In effect, the charged macromolecular surfaces are 'wired' by electrolyte pathways and pools. The electrolyte pathways provide a rationale for the low apparent diffusion coefficients and the heterogeneity of protein diffusion in the cytoplasm of bacteria [6,48]. In Figure 1, the macromolecular structure of the cytoplasm near the cell membrane shows only the slowly diffusing macromolecules, coupled with the electrolyte pathways that are connected to integral membrane proteins to make electrochemical contact with the extracellular milieu. Our model therefore links the classical membrane 'fluid mosaic' model (and its related chemiosmotic phenomena) with the transient structure of the cytoplasm.

Maxwellian switches: semi-conducting pathways

What is the nature of the electrolyte pathways? From a simplified theoretical viewpoint, two negatively charged surfaces and the electrolytes between them constitute an electrolyte pathway. The ionic distributions in the pathway depend on the geometry of the surfaces, on their

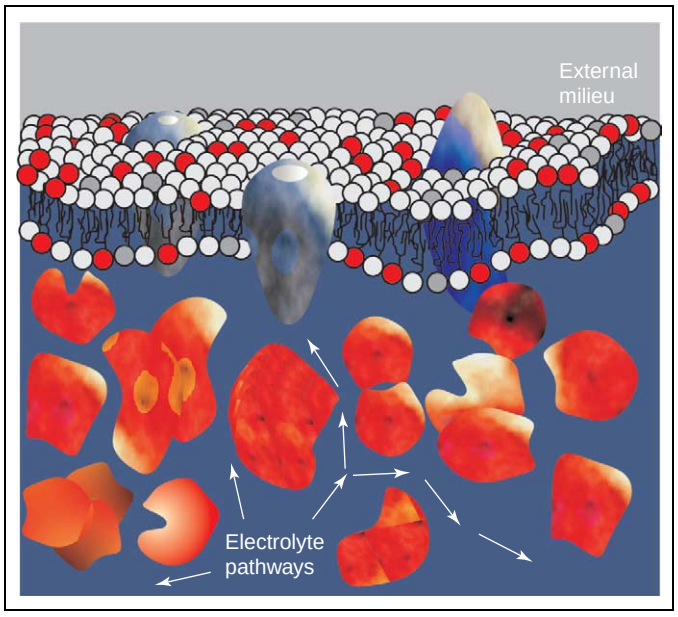

Figure 1. The region of the cytoplasm near the membrane surface. The electrolyte pathways are indicated by arrows. Proteins floating in the membrane bilayer are shown in blue, cytoplasmic proteins are shown in orange, and lipid headgroups are shown as red (anionic) and gray (neutral) circles.

charge densities and on the ionic strength of the pools with which the surfaces are equilibrated. According to the Maxwellian model of contiguous Poisson–Boltzmann equations [28–30], there exist 'Maxwellian switches' (electrostatic conditions) for transitions in the structure of ionic clouds near charged surfaces (Box 1).

Spitzer [28] has defined Maxwellian switches and the related distributions of ions for electrolyte pathways equilibrated with charged ions. For cytoplasmic ions, the pathways are equilibrated with a mixture of electrolytes containing monovalent and divalent anions. Examples include dihydrogen and monohydrogen phosphate (usually denoted by P_i), and biochemically complexed ions such as Mg-ADP$^-$ and Mg-ATP^{2-} in mixtures with singly charged ions such as glutamate and K^+. The Maxwellian switches and the related ionic distributions are described in Figure 2 and Table 1, which show that electrolyte pathways differ in their ability to pass or exclude anions. For example, a pathway could enable all ions to pass to variable degrees (Figure 2a–c); alternatively, only cations and monovalent anions might be allowed to pass (Figure 2d), or the pathways could exclude all anions (Figure 2e). We propose that such 'semi-conducting' pathways assist in the execution of logical operations: in other words, the 'output' (the ions that are forbidden to pass) is determined by the surface and middle electrostatic potentials (the 'set points'). In a way, the electrolyte pathways and their switches resemble semi-conducting silicon chips. Silicon chip pathways are hard-wired *in silico*, however, whereas cytoplasmic electrolyte pathways are always in (slow) thermal motion and readjust in response to environmental conditions *in vivo*.

Electrolyte pathways and vectorial metabolism

We can extend the analogy of a cell as a 'bustling city' and add that the postulated electrolyte pathways and pools

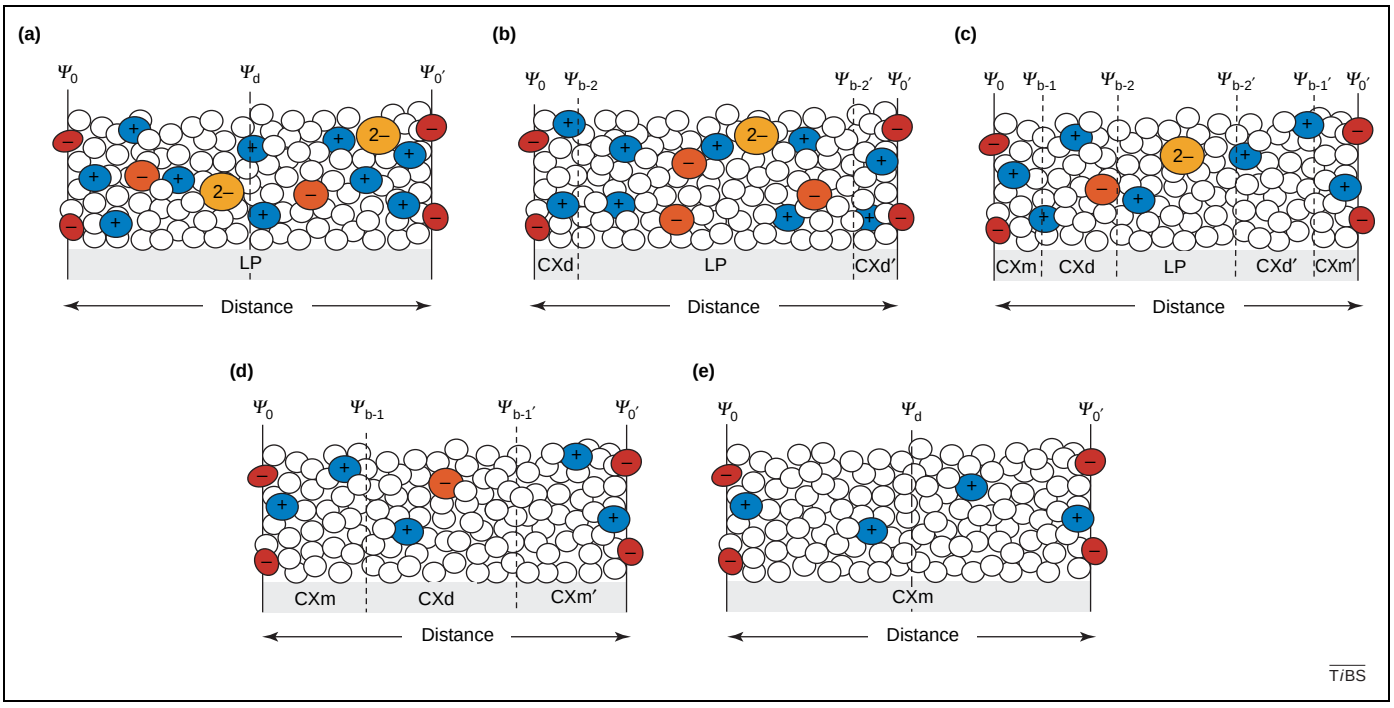

Figure 2. Two negatively charged surfaces form an electroneutral electrolyte pathway with water and ions. Water molecules are shown as white spheres and ions as colored spheres. When the pathway is equilibrated with a mixed salt solution of monovalent (red) and divalent (orange) anions and common cations (blue), the ionic distributions will have transitions, as defined by two Maxwellian switches (i.e. the co-ion exclusion boundaries: b_{-1} for monovalent anions, and b_{-2} for divalent anions). Boundary b_{-1} is at a potential of -25.7 mV$=kT/e$, and boundary b_{-2} is at potential -12.3 mV$=kT/(2e)$. The switches become operational as the negative electrostatic potential ψ_0 decreases as a result of increasing (negative) surface charge density **(a)**, decreasing ionic strength in the pools **(b)** or decreasing separation distance of the surfaces **(c)**, or combinations of these physicochemical variables. The low potential (LP) ionic distribution in (a) corresponds to the classical Debye–Hückel case without Maxwellian switches. In (b), the switch at b_{-2} is shown to be operational by the exclusion of divalent anions from the surface when the ionic strength drops (fewer ions shown). This switch prevents close-range interactions between free divalent anions and the negative surface in the divalent co-ion exclusion region denoted CXd. In (c), the second switch at b_{-1} becomes operational by the exclusion of monovalent anions from the surface region denoted CXm when the ionic strength drops further (fewer ions shown). When the ionic strength drops even further **(d)**, or the surface potential becomes more negative, the divalent co-ion boundaries b_{-2} from each surface become coincident and all of the divalent anions are removed from the pathway: in other words, the pathway admits only cations and monovalent anions. If the ionic strength decreases further **(e)**, or an even more negative surface potential develops, the monovalent co-ion boundaries b_{-1} from each surface become coincident and all of the monovalent anions are removed from the pathway: in other words, the pathway admits only cations. These five electrostatic transitions are summarized in Table 1.

represent the 'electrical grid' of such a city. This grid carries ionic metabolites that provide energy and information to the macromolecular complexes (machines) in the cytoplasm and in the membrane (Figure 1). The grid is being constantly expanded and 'rewired' during cell growth. It also responds to environmental insults, such as changes in cell volume caused by water efflux or influx during osmotic stress or extracellular conditions that interfere with pH homeostasis and alter the ionization state of metabolites and macromolecules [49]. In this sense, our cytoplasmic model conforms to Mitchell's ideas of vectorial metabolism across cellular membranes driven by electrochemical gradients [50]. The vectorial chemiosmotic reactions and transport processes in the membrane are linked to the vectorial metabolism of the cytoplasm, which takes place in the electrolyte pools and pathways (Figure 2).

The metabolic energy, obtained via respiration or light-harvesting reactions and stored in the membrane potential and pH gradient, is partly converted to ATP by the F_0F_1-ATP synthase and then distributed as ATP current via the electrical grid throughout the cytoplasm. For example, ADP-Mg^- and monovalent inorganic phosphate ions could be supplied by 'higher potential' pathways to the F_0F_1-ATP synthase, which would then release ATP-Mg^{2-} through a 'lower potential' pathway into a nearby pool. As the electrochemical ATP potential in such a pool rises, an increasing electrochemical gradient would develop with other cytoplasmic pools. The ATP ions could then flow via other pathways (and pools) to the pool with the lowest ATP potential. Other pathways might be set to higher potentials to become impassable to ATP, or they might be set to lower potentials to enable simultaneous distribution of ATP to more than one location. Thus, the ATP is distributed to other cytoplasmic and membrane-bound devices that consume ATP, such as ribosomes, biosynthetic enzymes and membrane transporters, among others.

Table 1. Electrostatic transitions in a pathway with negative surface charges and monovalent and divalent anions

Surface boundary	Mid-separation	Exclusion of anions from the pathway	Figure
$\Psi_0 > -12.3$ mV	$\Psi_d > -12.3$ mV	No co-ion exclusion (the Debye–Hückel case)	2a
$\Psi_0 = -12.3$ mV	$\Psi_d > -12.3$ mV	Partial exclusion of divalent anions begins	2b
$\Psi_0 = -25.7$ mV	$\Psi_d > -12.3$ mV	Partial exclusion of monovalent anions begins	2c
$\Psi_0 < -25.7$ mV	$\Psi_d = -12.3$ mV	Total exclusion of divalent anions	2d
$\Psi_0 < -25.7$ mV	$\Psi_d = -25.7$ mV	Total exclusion of monovalent anions	2e

Ψ_0, surface potential; Ψ_d, mid-point separation potential.

Concluding remarks

We propose that the crowded cytoplasm of many cells is organized by networks of electrolyte pathways and pools, which are transiently stabilized by screened electrostatic forces. These networks supply ionic metabolites and metabolic energy to membrane-embedded and intracellular molecular machines, and they respond to environmental stimuli through integral membrane proteins. The abstract metabolic and signaling pathways of classical biochemistry probably function through the proposed network of transient but tangible electrolyte pools and pathways. Contrary to membranes, where proteins and lipids can be observed in subdomains, so far there have been no experimental observations of the transient structure of the cytoplasm. But 'hard-wired', nanoscale electrolyte pathways in the 3–30 nm range could be constructed from surface-tethered charged macromolecules in order to study their semi-conducting, electrochemical and mechanical properties *in vitro*.

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